56. (Twice amended) The method of claim 38, wherein the cell [is genetically engineered to express] expresses [a]nucleic acid segments encoding 2-methylisocitrate dehydratase protein, a 2-methylisocitrate dehydratase protein, a 2-methylisocitrate dehydratase protein, a 2-methylisocitrate dehydratase protein, a 2-methylisocitrate lyase protein, a succinate:acetyl-CoA transferase protein, a succinate-semialdehyde dehydrogenase protein, and a 4-hydroxybutyrate dehydrogenase protein.

Remarks

Claims 38-61 and 64 are pending. Claims 38 and 52-56 have been amended. Claims 52-56 have been amended to provide fot a method wherein the cell expresses nucleic acid segments encoding enzymes (support can be found for example at lines 28-22, bridging pages 16-18). Claim 38 has been amended to require co-expression of the PHA synthase and the fatty acid-acyl Co A transferase under the control of a single promoter. Support is found on page 8, lines 13-15, and in examples 1 and 2.

This amendment should be entered since this issue has been raised and considered by the examiner previously. See the Amendment mailed September 13, 2001.

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 38-61 and 64 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention.

Claims 38-61 and 64 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled.

Claims 52-56 were rejected under 35 U.S.C. 112, although the rejection is improper, since it is

on the basis of added matter, not enablement or written description. Applicants respectfully traverse these rejections to the extent that it is applied to the claims as amended.

Enablement

The Examiner alleges that the specification does not reasonably provide enablement for a method of preparing a polyester using a PHA synthase gene and 4-HB Co-A transferase gene different form SEQ ID NO:1 and SEQ ID NO:2, respectively. The Examiner is relying on conclusory statements without putting forth specific reasons describing why the claims are not enabled by the specification. The Examiner cannot just assert that the claims are not enabled. As stated in *Re Marzocchi* at 439 F.2d 220 (C.C.P.A. 1971):

> It is incumbent upon the Patent Office, whenever a rejection on [enablement under § 112, first paragraph] is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the Applicant to go to the trouble and expense of supporting his presumptively accurate disclosure.

The M.P.E.P. instructs Examiners to make specific findings of facts to rebut the Applicants presumption and "specifically identify what information is missing and why one of skill in the art could not supply the information without undue experimentation." M.P.E.P. at § 2164.05. The Examiner should provide references to support a *prima facie* case of lack of enablement. *Id.*

The structures of the claimed PHA synthase and 4-HB CoA transferase are defined by the substrates recognized and subsequently converted to product, as well as by reference to the specification and the enormous body of literature referred to in the specification, for example at page 8, and as reviewed in the application at page 3, lines 9-20, as to the PHA synthase. A number of PHA synthases from different sources have been cloned, inserted into bacteria, yeast

and plants and expressed, where the expressed enzyme utilized substrate to produce a PHA (hence the name "PHA synthase"). The "enzymatic" nature of the claimed enzymes, in combination with the specific activity of the encoded protein, distinguishes the claimed enzymes from others. The identification of "substrates" is required for the determination of the structure of any enzyme that catalyzes a specific reaction. Methods are well established in the art that allow for the isolation of the genes and nucleic acid segments encoding the claimed enzymes, however, this is simply not necessary.

The standard under 35 U.S.C. 112 for enablement is whether one skilled in the art can make and use that which is claimed. Clearly this is the case - appropriate genes are available commercially and through the literature. Patents have been issued on the various enzymes (note, not restricted to any particular species) based on evidence showing that one skilled in the art can isolate the equivalent gene from other sources, using as a primer, for example, the gene sequence provided by applicants. One is **not** required to provide that which is already known and available.

Written Description

The standard under 35 U.S.C. 112, written description is whether the applicant has adequately described that which is claimed to demonstrate that he has possession of the claimed invention. Clearly this standard has also been met. The applicants have acknowledged that the individual enzymes from a variety of sources are known and described in the literature. Indeed, the examiner's position is that the **entire** invention is described in the literature! Moreover, applicants have demonstrated that they have reduced to practice that which is claimed, and

obtained better than expected results. The better-than-expected results were not the result of selection of some obscure or particular enzyme sequence, but the selection of the two enzymes, the PHA synthase and the fatty acid:acyl Co A transferase, which were placed in an appropriate host (also well known and commercially available to those skilled in the art), where the enzymes were expressed and polymer produced in high yield.

The examiner's attention is drawn to page 11 of the application for a list of available, known and useful PHA synthase enzymes.

The examiner's attention is drawn to page 11 of the application for a list of available, known and useful fatty acid:acyl Co A transferases.

There is NO legal requirement that applicants reduce to practice each enzyme or each gene to have support for a claim to a genus.

Example 1 at page 21 shows that *E. coli* were transformed with a nucleic acid segment encoding a PHA synthase and fatty acid:acyl Co A transferase, and page 22 shows that controls contained the enzymes alone or in a different position relative to the promoter. Example 2 on page 2 shows that 4-hydroxybutyric acid was produced and on page 24, that 4-HB-3-HB copolymers were produced through addition of appropriate substrates to the culture medium. Very high levels of polymer were accumulated.

This has apparently already been admitted by the Examiner, ("a variety of the two enzymes are described in the specification and in the art..."; Office Action mailed on May 3, 2002; see page 3).

No evidence has been provided by the examiner in support of the rejection, only argument. The Board of Appeals as well as the courts have repeatedly stated that this alone is not sufficient to maintain a rejection under 35 U.S.C.112, particularly when as is the case here, it is unclear even under what aspect of 112 the rejection is being made, although it is possible the rejection is being made under the premise of lack of written description (it certainly cannot be for lack of possession, in view of the numerous examples showing actual reduction to practice).

The Examiner's contradictory statements set aside, the Applicants respectfully remind the Examiner that the inquiry into adequate written description is not performed in a vacuum. "Knowledge of one skilled in the art is relevant to meeting [the written description] requirement." *Enzo Biochem, Inc. v. Gen-Probe*, Docket No. 01-1230 (Fed. Cir. Apr. 2, 2002) (slip op.). This fact has implications not only for validity challenges, but also for patent prosecution. *See In re Alton*, 76 F.3d 1168, 1174-75 (Fed. Cir. 1996). The term "fatty acid:acyl-CoA transferase" catalyzes an acyl group transfer according to the reactions depicted on page 5 (a well known and characterized reaction in the art). The substrates and products produced *via* the reactions define, structurally and functionally, the *only* enzyme that generates the specified acyl group transfer (i.e. fatty acid:acyl-CoA transferase). The same reasoning is applied to the term "polyhydroxyalkanoate (PHA) synthase". PHA synthases are defined by the catalytic activity that converts hydroxyacyl-CoAs to polyhydroxyalkanoates and free CoA. These two enzymes (PHA synthase and fatty acid:acyl-CoA transferase) are defined in the art by the

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reactions they catalyze. The substrates define their respective catalytic pockets and three-

dimensional shape. The products produced add to what is already a straightforward defining characteristic (types of substrate utilized).

The Applicants submit that one of skill in the art would find the generically claimed enzymes, described on the basis of the disclosure of the substrate/product reaction function and accessible structures (of, not only the enzymes, but the substrates as well), consistent with the PTO Guidelines. The binding of enzyme to substrate is analogous to two complementary nucleic acid sequence coming together to bind. The well known and characterized sequence of one complementary fragment defines the structure of the other, just as the well known and characterized structure of substrate and product define the enzyme that catalyzes the conversion of one (substrate) to the other (product).

It should be noted that one of ordinary skill in the art will readily appreciate that hydrogen bonding arrangements and hydrophobic interactions (i.e. chemical and physical properties) define the structure of the claimed enzymes in a way that provides one with a mental picture of a defined "space" that can only be accessed (or bound to) by a substrate of the correct "shape". It will help, perhaps, to view the claimed enzyme as a "lock" and the substrate as the "key", wherein the shape of the interior of the lock is defined by hydrophobic, hydrogen bonding, and electrostatic forces provided by the three-dimensional structure and energy constraints of the enzyme. The key (substrate) will only fit into the lock if it is able to "complement" these forces and bind to the enzyme, thereby defining the catalytic core of the enzyme.

New Matter

Claims 52-56 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

Page 5, lines 17-18, explicitly defines the term "heterologous". The claims have been amended to define cells expressing nucleotide segments encoding the claimed proteins.

The specification clearly states at page 4, lines 7-11, that recombinant vectors, cells containing nucleic acid segments, and methods for the preparation of polyester materials are among the reagents and protocols provided. It would be readily apparent to one of ordinary skill in the art that in view of the definition provided for the term "heterologous" and the teaching of recombinant vectors and cells containing nucleic acid segments, that cells containing the claimed proteins, provide for gene or nucleic acid segment expression thereby producing the claimed enzymes/proteins.

With respect to the specific enzymes, for 2-oxyglutarate decarboxylase protein and a heterologous 4-hydroxybutyrate dehydrogenase protein, support is found at page 18, lines 26-28; for succinate-semialdehyde dehydrogenase protein and a 4-hydroxybutyrate dehydrogenase, support is found at page 18, lines 23-26, and for 2-methylisocitrate dehydratase protein, a 2-methylisocitrate dehydratase protein, a 2-methylisocitrate dehydratase protein, a 2-methylisocitrate lyase protein, a succinate:acetyl-CoA transferase protein, a succinate-

semialdehyde dehydrogenase protein , and a 4-hydroxybutyrate dehydrogenase protein, support is found at page 18, line 29 to page page 19, line 1.

Rejection Under 35 U.S.C. § 102

Claims 38-42, 44-61 and 64 were rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 6,117,658 to Dennis *et al.* ("Dennis"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The Examiner asserts that Dennis teaches a cell comprising at least one recombinant sequence that encodes a PHA pathway and at least one recombinant sequence that encodes a succinic semialdehyde metabolic ***pathway***.

Dennis teaches providing cells with one or both ***intact*** pathways, whether recombinant, or not (column 6, lines 35-37). Dennis teaches PHA comprising 4HB monomer units can be produced utilizing cellular pathways other than the full succinyl-CoA pathway, ***as long as the pathway produces a succinic semialdehyde*** (see column 8, lines 41-44). The Examiner's assertion that a "*Clostridium kluyveri* orfZ 4-hydroxybutyric acid acyl-Co A transferase gene can be used as one of the sequences in addition to other genes", is a true statement ***if*** the Examiner intends orfZ's gene product to function as part of a pathway dedicated to the metabolism of succinic semialdehyde. Column 9, lines 21-39, states that all of the genes of the succinyl-CoA metabolic pathway are on a 7.5 kb DNA fragment. While the applicants obviously recognize that current genetic manipulation allows for separate expression of genes encoded within the segment, Dennis ***does not*** teach expression of a fatty acid:acyl CoA transferase separate from the

succinic semialdehyde metabolic pathway. Therefore Dennis does not disclose each of the claimed elements.

However, to facilitate prosecution, the claims have been amended to recite that the PHA synthase and fatty acid:acyl Co A transferase are on the same nucleotide molecule. Dennis does not show this feature. Therefore, Dennis does not disclose the subject matter of the claims as pending upon entry of the amendment.

Rejection Under 35 U.S.C. § 103

Claims 38, 39, and 41-43 were rejected under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 6,117,658 to Dennis *et al.* ("Dennis"), in view of U.S. Patent No. 5,512,468 to Greener ("Greener"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

Dennis is discussed above.

With respect to Greener, the Examiner has somehow provided the motivation to arrive at the claimed method in a certain type of E. coli cells based on the assertion that transformation is somehow functionally linked to the production of enzymes. Enclosed is a copy of the On-line Medical Dictionary definition of the term "genetic transformation" (see also column 1, lines 16-18, of Greener for a clear definition of "transformation"). The functional expression of a gene of interest is dependent upon events beyond the "unidirectional transfer and incorporation of foreign DNA by the cell". While nucleic acid uptake by the cell is required for the production of protein encoded by the DNA, proper transcriptional, translational, and post-translational apparatuses and mechanisms are required for the functional, and increased, production of the

enzymes and enzymatic activity encoded in the transforming nucleic acid. Therefore while the expression of functional alpha amylase or LacZ had been shown in XL-1 Blue cells (per the Greener disclosure), this is not predictive of the successful preparation of a polyester from recombinant cells expressing PHA synthase protein and fatty acid:acyl-coenzyme A transferase protein.

However, even if there were a disclosure of each of the claimed element, and the motivation to combine, with an expectation of success (which we do not concede), the method as defined by the amended claims produces unexpected results. The claims have been amended to recite that the synthase and transferase are on a single element. This results in substantially higher production of polymer than the introduction of the synthase and transferase on separate nucleotide molecules, as described by Dennis.

Referring to the examples, several plasmids were made as described in example 1:

Plasmids pKSSE5.3 and pSKSE5.3 contain the PHA synthase and transferase

Plasmids pKSSA35 and pSKSA35 contain the PHA synthase and thiolase

Plasmids pKSAE18 and pSKAE18 contain transferase only.

Example 2 demonstrates that putting both genes adjacent and co-linear with the promoter significantly increases polymer yield. This finding is completely unexpected and could not have been obvious from any of the cited art, alone or in combination.

Allowance of claims 38-61 and 64 is respectfully solicited.

Respectfully submitted,

Patrea L. Pabst
Reg. No. 31,284

Date: August 5, 2002

HOLLAND & KNIGHT LLP
One Atlantic Center, Suite 2000
1201 West Peachtree Street
Atlanta, Georgia 30309-3400
(404) 817-8473
(404) 817-8588 (Fax)

CERTIFICATE OF FACSIMILE TRANSMISSION UNDER 37 C.F.R. § 1.8

I hereby certify that this correspondence is being facsimile transmitted to the U.S. Patent and Trademark Office on August 5, 2002.

Patrea Pabst

Date: August 5, 2002

MARKED UP VERSION OF AMENDMENTS PURSUANT TO 37 C.F.R. § 1.121

Marked Up Version of Amended Claims

Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

38. (Twice Amended) A method for the preparation of a polyester, comprising the steps of: culturing recombinant cells under conditions suitable for the production of the polyester, wherein the recombinant cells [co-express a polyhydroxyalkanoic acid synthase protein and] have been genetically engineered to express a polyhydroxyalkanoic acid synthase and a fatty acid:acyl-coenzyme A transferase protein under the control of a single promoter.

39. The method of claim 38, wherein the cell is a plant cell, mammalian cell, insect cell, fungal cell, or bacterial cell.

40. The method of claim 39, wherein the cell is a plant cell.

41. The method of claim 39, wherein the cell is a bacterial cell.

42. The method of claim 41, wherein the cell is *Escherichia coli*.

43. The method of claim 42, wherein the bacterial cell is *Eschrichia coli* strain XL1-Blue.

44. The method of claim 38, wherein the polyhydroxyalkanoic acid synthase protein is a polyhydroxyalkanoic acid synthase protein from *Alcaligenes eutrophus*.

45. The method of claim 44, wherein the *Alcaligenes eutrophus* polyhydroxyalkanoic acid synthase protein is encoded by the *Alcaligenes eutrophus* polyhydroxyalkanoic acid synthase structural gene.

46. The method of claim 38, wherein the fatty acid:acyl-coenzyme A transferase protein is a ~~4-hydroxybutyrate:acyl-coenzyme A transferase protein.~~

47. The method of claim 46, wherein the 4-hydroxybutyrate:acyl-coenzyme A transferase protein is a *Clostridium kluyveri* 4-hydroxybutyrate:acyl-coenzyme A transferase protein.

48. The method of claim 47, wherein the *Clostridium kluyveri* 4-hydroxybutyrate:acyl-coenzyme A transferase protein is encoded by *Clostridium kluyveri orfZ* 4-hydroxybutyrate:acyl-coenzyme A transferase structural gene.

49. The method of claim 38, wherein the culture contains glucose.

50. (amended) The method of claim 38, wherein the culture contains materials selected from the group consisting of 4-hydroxybutyric acid, the sodium salt of 4-hydroxybutyric aicd, γ-butyrolactone, 1,4-butanediol, 4-hydroxyvaleric acid, γ-valerolactone, 1,4-pentanediol, 3-hydroxybutyric acid, the sodium salt of 3-hydroxybutyric acid, a hydroxypropionic acid, a hydroxybutyric acid, a hydroxyvaleric acid, a hydroxycaproic acid, a hyroxyheptanoic acid, a hydroxyoctanoic acid, a hydroxydecanoic acid, γ-caprolactone, γ-heptanolactone, γ-octanolactone, or γ-decanolactone.

51. The method of claim 38, wherein the culture contains molecular oxygen.

52. (Twice Amended) The method of claim 38, wherein the cell [is genetically engineered to express] expresses a heterologous nucleic acid segment encoding a protein capable of hydrolyzing a lactone to the corresponding hydroxyalkanoic acid.

53. (Twice Amended) The method of claim 38, wherein the cell [is genetically engineered to express] expresses [a]heterologous nucleic acid segments encoding 2-oxyglutarate decarboxylase protein and a heterologous 4-hydroxybutyrate dehydrogenase protein.

MARKED UP VERSION OF AMENDMENTS PURSUANT TO 37 C.F.R. § 1.121

54. (Twice amended) The method of claim 38, wherein the cell [is genetically engineered to express] expresses a heterologous nucleic acid segment encoding a protein selected from the group consisting of a 2-methylcitrate synthase protein, a 2-methylcitrate dehydratase protein, 2-methylisocitrate dehydratase protein, 2-methylisocitrate lyase protein, a succinate:aceyl-CoA transferase protein, a succinate-semialdehydc dehydrogenase protcin, and a 4-hydroxybutyrate dehydrogenase protein.

55. (Twice amended) The method of claim 38, wherein the cell [is genetically engineered to express] expresses [a]nucleic acid segments encoding succinate-semialdehyde dehydrogenase protein, and a 4-hydroxybutyrate dehydrogenase protein.

56. (Twice amended) The method of claim 38, wherein the cell [is genetically engineered to express] expresses [a]nucleic acid segments encoding 2-methylisocitrate dehydratase protein, a 2-methylisocitrate dehydratase protein, a 2-methylisocitrate dehydratase protein, a 2-methylisocitrate dehydratase protein, a 2-methylisocitrate lyase protein, a succinate:acetyl-CoA transferase protein, a succinate-semialdehyde dehydrogenase protein, and a 4-hydroxybutyrate dehydrogenase protein.

57. The method of claim 38, wherein the polyester is a homopolyester.

58. The method of claim 57, wherein the homopolyester is poly(4-hydroxybutyric acid).

59. The method of claim 57, wherein the homopolyester is poly(3-hydroxybutyric acid).

60. The method of claim 38, wherein the polyester is a copolyester.

61. The method of claim 60, wherein the copolyester is poly(3-hydroxybutyric acid –co-4-hydroxybutyric acid).

64. The method of claim 38 further comprising separating the polyester from the recombinant cells.

Clean Version of Amended Claims
Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

38. (Twice Amended) A method for the preparation of a polyester, comprising the steps of: culturing recombinant cells under conditions suitable for the production of the polyester, wherein the recombinant cells have been genetically engineered to express a polyhydroxyalkanoic acid synthase and a fatty acid:acyl-coenzyme A transferase protein under the control of a single promoter.

39. The method of claim 38, wherein the cell is a plant cell, mammalian cell, insect cell, fungal cell, or bacterial cell.

40. The method of claim 39, wherein the cell is a plant cell.

41. The method of claim 39, wherein the cell is a bacterial cell.

42. The method of claim 41, wherein the cell is *Escherichia coli*.

43. The method of claim 42, wherein the bacterial cell is *Eschrichia coli* strain XL1-Blue.

44. The method of claim 38, wherein the polyhydroxyalkanoic acid synthase protein is a polyhydroxyalkanoic acid synthase protein from *Alcaligenes eutrophus*.

45. The method of claim 44, wherein the *Alcaligenes eutrophus* polyhydroxyalkanoic acid synthase protein is encoded by the *Alcaligenes eutrophus* polyhydroxyalkanoic acid synthase structural gene.

46. The method of claim 38, wherein the fatty acid:acyl-coenzyme A transferase protein is a 4-hydroxybutyrate:acyl-coenzyme A transferase protein.

MARKED UP VERSION OF AMENDMENTS PURSUANT TO 37 C.F.R. § 1.121

47. The method of claim 46, wherein the 4-hydroxybutyrate:acyl-coenzyme A transferase protein is a *Clostridium kluyveri* 4-hydroxybutyrate:acyl-coenzyme A transferase protein.

48. The method of claim 47, wherein the *Clostridium kluyveri* 4-hydroxybutyrate:acyl-coenzyme A transferase protein is encoded by *Clostridium kluyveri orfZ* 4-hydroxybutyrate:acyl-coenzyme A transferase structural gene.

49. The method of claim 38, wherein the culture contains glucose.

50. (amended) The method of claim 38, wherein the culture contains materials selected from the group consisting of 4-hydroxybutyric acid, the sodium salt of 4-hydroxybutyric aicd, γ-butyrolactone, 1,4-butanediol, 4-hydroxyvaleric acid, γ-valerolactone, 1,4-pentanediol, 3-hydroxybutyric acid, the sodium salt of 3-hydroxybutyric acid, a hydroxypropionic acid, a hydroxybutyric acid, a hydroxyvaleric acid, a hydroxycaproic acid, a hyroxyheptanoic acid, a hydroxyoctanoic acid, a hydroxydecanoic acid, γ-caprolactone, γ-heptanolactone, γ-octanolactone, or γ-decanolactone.

51. The method of claim 38, wherein the culture contains molecular oxygen.

52. (Twice Amended) The method of claim 38, wherein the cell expresses a heterologous nucleic acid segment encoding a protein capable of hydrolyzing a lactone to the corresponding hydroxyalkanoic acid.

53. (Twice Amended) The method of claim 38, wherein the cell expresses heterologous nucleic acid segments encoding 2-oxyglutarate decarboxylase protein and a heterologous 4-hydroxybutyrate dehydrogenase protein.

MARKED UP VERSION OF AMENDMENTS PURSUANT TO 37 C.F.R. § 1.121

54. (Twice amended) The method of claim 38, wherein the cell expresses a heterologous nucleic acid segment encoding a protein selected from the group consisting of a 2-methylcitrate synthase protein, a 2-methylcitrate dehydratase protein, 2-methylisocitrate dehydratase protein, 2-methylisocitrate lyase protein, a succinate:aceyl-CoA transferase protein, a succinate-semialdehyde dehydrogenase protein, and a 4-hydroxybutyrate dehydrogenase protein.

55. (Twice amended) The method of claim 38, wherein the cell expresses nucleic acid segments encoding succinate-semialdehyde dehydrogenase protein, and a 4-hydroxybutyrate dehydrogenase protein.

56. (Twice amended) The method of claim 38, wherein the cell expresses nucleic acid segments encoding 2-methylisocitrate dehydratase protein, a 2-methylisocitrate dehydratase protein, a 2-methylisocitrate dehydratase protein, a 2-methylisocitrate lyase protein, a succinate:acetyl-CoA transferase protein, a succinate-semialdehyde dehydrogenase protein, and a 4-hydroxybutyrate dehydrogenase protein.

57. The method of claim 38, wherein the polyester is a homopolyester.

58. The method of claim 57, wherein the homopolyester is poly(4-hydroxybutyric acid).

59. The method of claim 57, wherein the homopolyester is poly(3-hydroxybutyric acid).

60. The method of claim 38, wherein the polyester is a copolyester.

61. The method of claim 60, wherein the copolyester is poly(3-hydroxybutyric acid –co-4-hydroxybutyric acid).

64. The method of claim 38 further comprising separating the polyester from the recombinant cells.